CHAPTER 7

Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications

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Diabetes Care 2008; 31:517-21

ABSTRACT

Introduction Skin autofluorescence (AF) is a noninvasive measure of the level of tissue accumulation of advanced glycation endproducts, representing cumulative glycemic and oxidative stress. Recent studies have already shown a relationship between skin AF and diabetic complications, and its predictive value for total and cardiovascular mortality in type 2 diabetes mellitus. Our aim was to investigate the predictive value of skin AF for the development of microvascular complications in type 2 diabetes mellitus.

Methods At baseline, skin AF of 973 well-controlled type 2 diabetes patients was noninvasively measured with an AF reader. The aggregate clinical outcome was defined as the development of any diabetes associated microvascular complication of 881 surviving patients which was assessed at baseline and at the end of follow-up. Single endpoints were the development of diabetes associated retinopathy, neuropathy and (micro)albuminuria.

Results After a mean follow-up period of 3.1 years, baseline skin AF was significantly higher in patients who developed any microvascular complication, neuropathy and (micro)albuminuria, but not in those who developed retinopathy. Multivariate analyses showed skin AF as a predictor for the development of any microvascular complication along with HbA1c; for the development of neuropathy along with smoking, and for the development of (micro)albuminuria together with gender, HbA1c and diabetes duration. Skin AF did not have predictive value for the development of retinopathy, albeit diabetes duration did.

Conclusion Our study is the first observation of skin AF measurement as an independent predictor for the development of microvascular complications in type 2 diabetes mellitus.

INTRODUCTION

Hyperglycaemia, individual susceptibility and life-style are three key factors, which play an important role in the development of microvascular disease in diabetes

mellitus. One of the consequences of hyperglycaemia and attendantly increased generation of free radicals is the increased formation of advanced glycation endproducts (AGEs), besides the increased polyol and hexosamine fluxes, and activation of protein kinase C, which all contribute to tissue damage in diabetes [1,2]. Those AGEs can be described as the final products of slowly occurring non-enzymatic glycation of proteins that form cross-links with long-lived proteins such as collagen (the so called Maillard reaction). They may also accumulate as a result of oxidative stress related glycoxidation and lipoxidation pathways.

In the DCCT, intensive treatment as compared with conventional treatment showed that long term intensive treatment of hyperglycaemia in type 1 diabetic patients improved glycemic control, and delayed the progression of microvascular complications [3]. The UKPDS and other prospective studies have also shown an association between hyperglycaemia and increased risk of microvascular complications in type 2 diabetes [4-6]. The DCCT skin collagen ancillary study group showed the association of long-term intensive treatment of hyperglycaemia, as compared with conventional treatment, with lower levels of AGEs in skin collagen and they showed that these AGE levels in skin biopsies predicted the risk of development or progression of microvascular disease in type 1 diabetes mellitus, even after adjustment for HbA1c [7,8].

A newly described noninvasive method to assess tissue AGEs concerns skin autofluorescence (AF). This method is based on the specific fluorescence characteristics of AGEs and has been validated against specific AGE levels in skin biopsies in patients with diabetes or on hemodialysis, and in healthy controls [9,10].

Recently, the relationship between skin AF, reflecting AGE accumulation, and outcome has been studied in type 2 diabetes. Besides its relation with chronic complications of diabetes (in cross-sectional analyses), skin AF has also shown its independent predictive value for cardiovascular mortality and morbidity in patients with type 2 diabetes, and in patients with end-stage renal disease undergoing hemodialysis [10-12].

In this study, we analyzed whether skin AF, as a marker of AGE accumulation, can predict the development of microvascular complications in a type 2 diabetes population.

METHODS

Patients. Between May 2001 and May 2002, 973 primary care type 2 diabetes patients were included in the study cohort and had a skin AF measurement. The included patients were all participating in a shared-care project of the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC)-study and have also been described elsewhere [11]. During follow-up, data of 967 patients were analyzed for this study (6 patients were lost to follow-up). Eighty-six patients died before the end of follow-up and this subgroup will be addressed separately from the surviving 881 patients. Patients with a Fitzpatrick class V-VI skin type were excluded, because of the limitation of the autofluorescence reader (AFR) to measure accurately in dark skin types [13-15]. All participating patients visited the outpatient clinic at least once a year. Follow-up ended at January 2005. All of the included patients had given their informed consent, and approval by the local ethical committee had been obtained.

Skin autofluorescence. The AFR (prototype of the current AGE Reader; DiagnOptics BV, Groningen, the Netherlands), which measures skin AF, illuminates a skin surface of ~4 cm^2, guarded against surrounding light, with an excitation light source with peak intensity at ~370 nm. Emission light and reflected excitation light from the skin are measured with a spectrometer in the 300-600 nm range, using a glass fiber. AF was computed by dividing the average light intensity of the emission spectrum 420-600 nm by the average light intensity of the excitation spectrum 300-420 nm, multiplied by hundred and expressed in arbitrary units (a.u.). Skin AF of all patients was assessed at the volar side of the arm, 10 cm below the elbow fold. Six diabetes specialist nurses did the AF measurements with 2 identical AFR devices. The AFR has been validated and more extensively been described in previous studies [9,11].

Data collection. Clinical data and laboratory results were obtained at the time of the baseline skin AF measurement. Serum creatinine, nonfasting lipids (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides), and urinary albumin and creatinine were measured according to the standard laboratory procedures. HbA1c was measured with a Primus CLC-385 using boronate affinity chromatography and high-performance liquid chromatography (reference value 4.0 – 6.0%). Blood pressure measurement was a single measurement obtained after 5 minutes rest, with the patient in seated position, and using an aneroid device. At each visit to the outpatient clinic, and at the end of follow-up, the absence or presence of retinopathy, neuropathy and (micro)albuminuria were assessed.

Clinical endpoints. The aggregate clinical endpoint was the development of any diabetes associated microvascular complication, which was defined as the presence of at least one of the following diabetic complications: retinopathy, neuropathy and/or (micro)albuminuria, according the ADA Diabetes definitions [16]. The single clinical endpoints were described as the development of retinopathy, neuropathy or (micro)albuminuria. Retinopathy was determined by an ophthalmologist based on retinal photography. Presence of at least background retinopathy was assumed to imply retinopathy. Neuropathy was examined using a 5.07/10-g Semmes-Weinstein monofilament, applied on the dorsum of both feet at three different, non-callused areas (first toe, and first and fifth distal metatarsal bone). Neuropathy was considered in case of diminished sensibility, which was defined in case of at least two incorrect responses after 3 applications at each area (2 real and 1 false application) [17,18]. (Micro)albuminuria at baseline was defined as an albumin-to-creatinine ratio >2.5 mg/mmol for men and >3.5 mg/mmol for women in 2 subsequent urine samples or once in the year before baseline while using an ACE-inhibitor at baseline [19]. Newly developed (micro)albuminuria at follow-up was defined as an albumin-to-creatinine ratio >2.5 mg/mmol for men and >3.5 mg/mmol for women in 2 urine samples (one in the year before and one at the moment during follow-up) or an abnormal level of the albumin-to-creatinine ratio in the year before the end of follow-up whilst using an ACE-inhibitor at follow-up.

Statistical analysis

Oneway ANOVA using posthoc multiple comparisons (with Bonferroni correction) was used to compare mean skin AF between subgroups of microvascular complications in the 881 surviving patients. Subgroups are: A. no microvascular complication at baseline nor at follow-up. B. no microvascular complication at baseline, but a microvascular complication at follow-up. C. a microvascular complication at baseline and at follow-up.

Univariate and multivariate multinominal regression analyses were performed to determine the relationship of skin AF to the presence or development of microvascular disease. Patients without signs of microvascular complications at baseline nor at follow up were the reference categories in these calculations. In the multivariate analyses we controlled for potential confounding risk factors for the development of microvascular complications which were derived from the UKPDS findings, thereby including: gender, diabetes duration, HbA1c, current smoking, systolic blood pressure, HDL cholesterol, LDL cholesterol and triglycerides with the addition of BMI [4].

Odds-ratios (OR) and confidence intervals (95%) for skin AF were calculated in the univariate and in the multivariate analyses; p-values <0.05 were considered to be statistically significant.

RESULTS

The baseline characteristics of the surviving study population including mean skin AF of the total group are shown in *Table 1*. Mean age of our study population was 66 years, 46% male, with a relatively short median diabetes duration of 4.0 years and an interquartile range between 1.5 and 8.1 years. Eighty-five percent of this well-controlled diabetic study population was on a diet and/or oral agents; the other 15% of patients received insulin or combined insulin/oral agent treatment. In the 881 survivors, the prevalence of retinopathy, neuropathy and (micro)albuminuria at baseline was 19%, 24% and 24% respectively, resulting in an overall percentage of patients with a diabetes associated microvascular complication of 50%.

Table 1. Characteristics of the type 2 diabetic patients

Characteristic	*n=881*
Age in years	66 (11)
Gender (M/F)	406/475
Smoking (%)	19
BMI (kg/m²)	29.4 (4.8)
Systolic blood pressure (mmHg)	146 (20)
Diabetes duration (years)	4.0 (1.5-8.1)*
HbA1c (%)	6.6 (6.0-7.6)*
Creatinine (µmol/l)	95 (19)
Creatinine clearance (Cockcroft-formula) (ml/min)	77 (27)
Urinary albumin-to-creatinine ratio	1.41 (0.76-3.79)*
Total cholesterol (mmol/l)	5.2 (1.0)
HDL cholesterol (mmol/l)	1.3 (0.3)
LDL cholesterol (mmol/l)	2.9 (0.9)
Triglycerides (mmol/l)	2.1 (1.4-2.9)*
Microvascular disease (%)	50
Retinopathy (%)	19
Neuropathy (%)	24
(Micro)albuminuria (%)	24
Macrovascular disease (%)	37
Skin autofluorescence (total group) (a.u.)	2.74 (0.7)

Values are expressed as mean (SD). *Median and Interquartile range.

Table 2 shows the mean baseline skin AF of the 881 survivors subdivided in groups with continued absence or presence or the development of microvascular complications at follow-up. During a median follow-up period of 3.1 years, 61 patients (7.0%) developed retinopathy; their baseline skin AF did not differ from skin AF levels of patients who did not show or already had retinopathy at baseline. However, skin AF was higher in the patient groups who developed neuropathy or (micro)albuminuria compared to those without these complications. At follow-up newly developed neuropathy was diagnosed in 7.5% and newly developed (micro)albuminuria was found in 10.1%; 12.5% of the population developed at least one microvascular complication. Skin AF at baseline was also significantly higher in the patient groups who developed any microvascular complication or who already had a microvascular complication at baseline, compared to those patients who did not develop any microvascular disease at all.

Table 2. Mean skin AF (SD) at baseline and mean differences between groups

Microvascular complication		A= t_0 : absent t_{fu}: absent	B= t_0 : absent t_{fu}: present	C= t_0 : present t_{fu} : present		B vs. A	C vs. A	C vs. B
Retinopathy								
	AF	2.69 (0.73)	2.88 (0.74)	2.91 (0.72)	**ΔAF**	0.20	0.22	0.02
	n	647	61	169	**95% CI**	-0.04-0.43	0.07-0.37	-0.24-0.29
					p	0.14	0.002	1.00
Neuropathy								
	AF	2.67 (0.72)	2.93 (0.75)	2.88 (0.75)	**ΔAF**	0.26	0.21	-0.05
	n	596	66	215	**95% CI**	0.03-0.49	0.07-0.35	-0.29-0.20
					p	0.019	0.001	1.00
(Micro)albuminuria								
	AF	2.62 (0.68)	2.91 (0.67)	2.97 (0.83)	**ΔAF**	0.28	0.34	0.06
	n	570	87	207	**95% CI**	0.09-0.48	0.20-0.48	-0.16-0.28
					p	0.002	<0.001	1.00
Any								
	AF	2.52 (0.69)	2.86 (0.66)	2.88 (0.75)	**ΔAF**	0.34	0.36	0.01
	n	322	109	441	**95% CI**	0.15-0.53	0.23-0.48	-0.17-0.20
					p	<0.001	<0.001	1.00

Data are means (SD) of skin AF in arbitrary units within the group, and mean differences (ΔAF) between groups calculated with ANOVA (95% Confidence interval) with Bonferroni correction; t_0, baseline; t_{fu}, follow-up

Multinominal logistic regression analysis showed that skin AF was a strong predictor of the development of the aggregate of microvascular complications (OR 2.05 [1.51-2.80], p<0.001. Skin AF was significantly associated with the development of retinopathy (OR 1.42 [1.01-1.99], p=0.042), neuropathy (OR 1.59 [1.15-2.19], p=0.005), and (micro)albuminuria (OR 1.73 [1.28-2.34], p<0.001). After correction for the confounding risk factors, baseline skin AF still appeared to be significantly associated with the development of these endpoints, except for retinopathy (OR 1.21 [0.83-1.74], p=0.32), *Table 3*. Diabetes duration at baseline was the only significant independent variable for the development of retinopathy in this multivariate analysis (OR 1.10 [1.06-1.15], p<0.001). Surviving smokers developed less often neuropathy compared to non-smokers. In the non-surviving group (86 patients) 70% had a microvascular complication at baseline; there were 23 non-surviving smokers. Seventy percent of the non-surviving smokers already had a microvascular complication at

baseline and 13% of the non-surviving smokers developed a microvascular complication before they died.

When baseline skin AF levels are categorized in subgroups of practically feasible levels of skin AF (3 categories in rounded tertiles: skin AF < 2.35 a.u.; 2.35 ≤ skin AF < 3.00 a.u.; skin AF ≥ 3.00 a.u.), patients in the category skin AF ≥3.00 a.u do have a higher chance to develop a microvascular complication compared to patients with a lower skin AF level (*Table 4*).

Table 3. Variables related to the development of microvascular complications in type 2 diabetes mellitus by multinominal logistic regression analysis

	Any microvascular complication		Retinopathy		Neuropathy		(Micro)albuminuria	
Variables	*p value*	OR (95%CI)	*p value*	OR (95% CI)	*p value*	OR 95% CI	*p value*	OR 95% CI
Skin AF	<0.001	2.02 (1.45-2.81)	0.32	1.21 (0.83-1.74)	0.026	1.50 (1.05-2.14)	<0.001	1.88 (1.36-2.61)
Gender	0.02	0.55 (0.33-0.90)	0.91	0.97 (0.53-1.75)	0.78	1.09 (0.61-1.93)	0.001	0.42 (0.25-0.71)
HbA1c	0.004	1.30 (1.09-1.55)	0.13	1.18 (0.95-1.45)	0.87	1.02 (0.82-1.26)	0.034	1.21 (1.01-1.44)
Diabetes duration	0.66	1.01 (0.96-1.06)	<0.001	1.10 (1.06-1.15)	0.032	1.04 (1.00-1.08)	0.04	0.95 (0.90-0.997)
Smoking	0.07	0.56 (0.29-1.05)	0.09	0.48 (0.21-1.11)	0.011	0.29 (0.11-0.75)	0.96	1.02 (0.56-1.85)
Systolic BP	0.43	1.01 (0.99-1.02)	0.39	1.01 (0.99-1.02)	0.49	1.01 (0.99-1.02)	0.18	1.01 (0.996-1.02)
LDL cholesterol	0.48	1.09 (0.85-1.40)	0.66	0.93 (0.69-1.27)	0.35	0.87 (0.64-1.17)	0.30	1.15 (0.89-1.49)
HDL cholesterol	0.26	0.62 (0.27-1.43)	0.36	0.63 (0.23-1.70)	0.081	0.41 (0.15-1.12)	0.40	0.38 (0.15-0.96)
Triglycerides	0.54	0.94 (0.78-1.14)	0.41	0.91 (0.72-1.15)	0.85	0.98 (0.79-1.22)	0.19	0.87 (0.71-1.07)
BMI	0.27	1.03 (0.98-1.08)	0.33	1.03 (0.97-1.09)	0.56	0.98 (0.93-1.04)	0.39	1.02 (0.97-1.08)

Table 4. Newly developed microvascular complications subdivided in 3 skin AF-groups

n	*Microvascular complication*	*Skin AF <2.35 a.u.*	*2.35 ≤ Skin AF < 3.00 a.u.*	*Skin AF ≥ 3.00 a.u*
708	Retinopathy	15/241 (6.2)	18/251 (7.2)	28/216 (13.0)
662	Neuropathy	11/219 (5.0)	27/247 (10.9)	28/196 (14.3)
65	(Micro)albuminuria	18/225 (8.0)	31/253 (12.3)	38/179 (21.2)
431	Any	23/161 (14.3)	41/167 (24.6)	45/103 (43.7)

Number (%) of newly developed microvascular complications of subgroups; *n* is number of patients who did not have the complication at baseline

DISCUSSION

Our study provides the first evidence that skin AF is an independent predictor of the development of microvascular complications in a well-controlled type 2 diabetes population. Separately, this also holds for the development of neuropathy and (micro)albuminuria (and in univariate analysis for retinopathy). This noninvasive marker of tissue AGE accumulation may reflect the deleterious effects of long-term glycemic and oxidative stress. It was recently shown that skin AF is a predictor of 5-year coronary heart disease and mortality in diabetes [12]. The present study showed that skin AF also has a predictive value for the development of microvascular complications, which in the analysis of this study is superior to that of many other commonly used risk predictors like diabetes duration and HbA1c in type 2 diabetes. This conclusion is applicable for primary care type 2 diabetes patients, treated according to current standards, which is the large majority of type 2 diabetes patients in the Netherlands.

The DCCT/EDIC substudy already showed the predictive value for skin AGE levels obtained from skin biopsies for the progression of microvascular complications in patients with type 1 diabetes [8]. Our study population consisted of type 2 diabetes patients, with skin AGE level assessment by means of a noninvasive, rapid method. Another difference is that the DCCT-substudy investigated the development as well as the progression of microvascular complications. The limited follow-up period, the low rate of clearly classifiable progression of the microvascular complications, especially retinopathy, and the confounding role of introduced medication made us decide to restrict our study to the evaluation of the development of microvascular complications and not to address progression of these diabetic complications.

In retinopathy, skin AF turned out to have no prognostic value in the multivariate analysis. Possible explanations are the short follow-up period and the smaller amount of patients who developed retinopathy compared to the development of the other complications. Moreover the different pathophysiologic mechanisms of microvascular damage in the different organs (retina, kidneys and neurons) could play a role in the differences in incidence rates of outcomes. In particular, the pathobiology of

retinopathy might be different from those in the kidney and neurologic system due to a different role of vascular endothelial growth factor (VEGF) as a possible mediator for proliferation [20].

(Micro)albuminuria is an early clinical sign of diabetic nephropathy; when left untreated it predicts a high risk for the development of progressive renal damage which will eventually may lead to end stage renal disease. Progressive renal disease is also associated with a vastly increased cardiovascular risk. This study defined (micro)albuminuria as a sign of microvascular complication with the intention to reflect early stages of diabetic nephropathy.

In the predictive analyses the non-surviving patients were excluded from the analyses. These non-survivors had markedly increased skin AF values, but they also had a very high prevalence of microvascular complications at baseline (70%), so this does not reduce the strength of the relation between skin AF and microvascular complications at all.

Ethnicity is one of the mentioned UKPDS confounding risk factors for the development of microvascular disease. Because of the limitation of measuring skin AF in dark skin types, with the prototype of the AGE reader used in the present study, people with dark skin had to be excluded. Over 95% of the participants were Caucasian, therefore ethnicity was not taken into account in the analyses. Further developments of the AGE reader may hopefully enable measurements in dark skin type in future investigations.

Lutgers et al. previously described the other limitations of the AFR as a marker of tissue AGE accumulation: non-fluorescent AGEs will not be measured with the AFR, and other tissue components which fluoresce in the same range of wavelength might be confounders [11].

In conclusion, our study confirms skin autofluorescence as a helpful clinical method to identify type 2 diabetes patients who are at risk for (developing) any microvascular complication, neuropathy and (micro)albuminuria. Further investigation with longer follow-up needs to be done to assess whether or not skin autofluorescence is a factor in the development of diabetic retinopathy, and to assess the relationship of skin AF and

the progression of microvascular complications. Its non-invasive and time-saving application makes the AFR an easy clinical tool useful in the out-patient clinic in the risk assessment as well as for monitoring changes in accumulation of tissue AGEs reflecting long term glycemic stress.

REFERENCES

1. Smith U, Laakso M, Eliasson B, Wesslau C, Borén J, Wiklund O, Attvall S. Pathogenesis and treatment of diabetic vascular disease – illustrated by two cases. J Int Med 260: 409 – 420, 2006
2. Brownlee M. The pathobiology of diabetec complications. A unifying mechanism. Banting Lecture 2004. Diabetes 54: 1615 – 1625, 2005
3. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977 – 986, 1993
4. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner R, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 321:405 – 412, 2000
5. Klein R, Klein BE, Moss SE. Relation of glycemic control to diabetic microvascular complications in diabetes mellitus. Ann Intern Med 124: 90 – 96, 1996
6. Pirart J. Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973 (part 1). Diabetes Care 1: 168 – 88, 1978
7. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, Cleary PA, Lachin J, Genuth S: Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetec complications: DCCT Skin Collagen Ancillary Study Group: Diabetes Control and Complications Trial. Diabetes 48:870 – 880, 1999

8. Genuth S, Sun S, Cleary PA, Sell DR, Dahms W, Malone J, Sivitz W, Monnier VM, for the DCCT Skin Collagen Ancillary Study Group: Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and compliations participants with type 1 diabetes. Diabetes 54:3103 – 3111, 2005
9. Meerwaldt R, Graaff R, Oomen PHN, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans ROB, Smit AJ. Simple non-invasive assessment of advanced glycation endproduct accumulation. Diabetologia 47:1324 – 1330, 2004
10. Meerwaldt R, Hartog JWL, Graaff R, Huisman RJ, Links TP, den Hollander NC, Thorpe SR, Baynes JW, Navis G, Gans ROB, Smit AJ. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. J Am Soc Nephrol 16:3687 – 3693, 2005
11. Lutgers HL, Graaff R, Links TP, Ubink-Veltmaat LJ, Bilo HJ, Gans RO, Smit AJ. Skin autofluorescence as a non-invasive marker of vascular damage in patients with type 2 diabetes mellitus. Diabetes Care 29:2654 – 2659, 2006
12. Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans ROB, Smit AJ. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. Diabetes Care 30:107 – 112, 2007
13. Fitzpatrick TB. Soleil et peau. J Med Esthet 2:33 – 34, 1975
14. Fitzpatrick TB. The validity and practicability of sun-reactive skin types I trough VI. Arch Dermatol 124:869 – 871, 1988
15. Kawada A. Risk and preventive factors for skin phototype, review. J Dermatol Sci. 23 Suppl 1:S27 – 29, 2000
16. http://www.diabetes.org/diabetesdictionary
17. Rutten GEHM, De Grauw WJC, Nijpels G, Goudswaard AN, Uitewaal PJM, Van der Does FEE, Heine RJ, Van Ballegooie E, Verduijn MM, Bouma M. Diabetes mellitus type 2. Huisarts en Wetenschap 49:137 – 152, 2006
18. Valk GD, de Sonnaville JJ, van Houtum WH, Heine RJ, van Eijk JT, Bouter LM, Bertelsmann FW. The assessment of diabetic polyneuropathy in daily clinical practice: reproducibility and validity of Semmes-Weinstein monofilaments

examination and clinical neurological examination. Muscle&Nerve 20:116 – 118, 1997

19. NIV-CBO Richtlijn diabetische nefropathie: monitoring, diagnostiek en behandeling. ISBN-10: 90-8523-138-8, 2006
20. Aiello LP and Wong JS. Role of vascular endothelial growth factor in diabetic vascular complications. Kidney Int 58 Suppl 7:S113 – 119, 2000